HIGH-TEMPERATURE–SHORT-TIME STERILIZED EVAPORATED MILK. IV. THE RETARDATION OF GELATION WITH CONDENSED PHOSPHATES, MANGANOUS IONS, POLYHYDRIC COMPOUNDS, AND PHOSPHATIDES

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SUMMARY

Polyphosphates, manganous salts, polyhydric compounds, and phosphatides are important additives for prolonging the storage life of high-temperature–short-time sterilized milk concentrates. A sixfold and threefold increase in the storage life of 3 to 1 and 2 to 1 concentrates was, respectively, obtained by adding per 100 g milk 0.05 g sodium polyphosphate containing an average of 4.8 P atoms per chain; and a similar increase was obtained by adding approximately the same quantity of $MnSO_4$. In concentrations greater than about 1 g per 100 g milk solids, the polyphosphate conduced to age-thickening. The polyphosphates function also as stabilizers against heat coagulation during sterilization. The antigelation activity of the polyphosphates increased with increasing concentration and chain length. The cyclic tetrametaphosphate and adenosine triphosphate were more effective than the corresponding linear polymer and sodium tripolyphosphate, respectively.

Added orthophosphates were found to conduce to gelation.

Nearly a twofold increase in storage life was brought by the incorporation in 2 to 1 concentrates of 9.6 g per 100 g product of either lactose, sucrose, dextrose, or sorbitol. At higher concentration levels specific effects were met which limited the activity of the sugars, but not that of sorbitol. In stabilizing the lipid phase, added phosphatides stabilize HTST sterilized milk concentrates both against heat coagulation during sterilization and gelation during storage.

Theoretical considerations postulating the existence of two kinds of micellar structures, one active in gelation phenomena, the other in heat coagulation, served as a guide in the selection of the additives used in the present study.

Gelation represents a single aspect of a many-sided instability problem encountered in the manufacture and storage of sterile milk concentrates. It is unique in the sense that only in the storage of high-temperature–short-time (HTST) sterilized milk products does it assume serious proportions. Upon the solution of the gelation problem may depend the successful application of HTST sterilization techniques to the development of improved fluid milk concentrates. The study of additives constitutes an inviting and obvious way to approach the problem as demonstrated by well-known observations on the ability of additives to retard coagulation during sterilization of conventionally prepared evaporated milk.

Studies in our laboratory, over a period of years and restricted to technologically applicable compounds, have disclosed the value of four groups of substances—polyphosphates, manganous salts, polyhydric compounds, and phosphatides—all of which, to some significant degree, possess the capacity of prolonging the storage life of HTST sterilized milk concentrates.

It is the object of this paper to report on the results of our studies, emphasizing for the moment their practical rather than their theoretical value.

METHODS AND MATERIALS

Methods. The techniques described in Parts 1 and 2 of this series of papers were used throughout the experiment (10, 11). With the exception of high-pressure homogenization, carried out with a Manton-Gaulin high pressure

homogenizer [1] capable of homogenizing at 8,000 psi, all operations were carried out on a laboratory scale. Homogenization was usually carried out at 160 F in two stages—7,500 and 500 psi. Forewarming for 17 min at the temperature of boiling water was carried out under nitrogen in a rotating film evaporator. Concentration under vacuum was effected in the same evaporator to a point slightly greater than the desired concentration, and final adjustment was made by the addition of a weighed quantity of water to the weighed concentrate. The concentrates sealed in bomb microviscometers were sterilized in an oil bath for either 5 or 15 sec at 137.4 C, then cooled in a water bath to room temperature. Samples were stored at 30 C in the same viscometers. Sedimentation and creaming were minimized by mounting the viscometers with their axes horizontal in a rotating device. Prior to a viscosity measurement, the position of the liquid thread in the viscometer was reversed twice in a centrifuge. Each viscometer tube contained a glass bead, and thus it could be arranged to function in the manner of the Höppler falling ball viscometer.[1] Viscosity measurements were made periodically on the same samples in triplicate throughout the experimental period. Fat and solids were determined according to the procedure recommended by Mojonnier and Troy (14), and homogenization indices by turbidimetry (2).

A plastometer was available consisting of a turntable mounted on a variable-speed, quick-starting motor. The viscometer tubes in radial grooves could be subjected to a relative centrifugal force varying from zero to 35 times gravity.

Many, but not all, procedural details may be inferred by referring to data in the various tables. In experiments with different condensed phosphates, additive concentrates were adjusted for comparison purposes so that, at each concentration level, the milks contained the same number of available orthophosphate groups (19).

Unless stated otherwise, in experiments with condensed phosphates and manganous salts, the additive in solution was added to the concentrate just prior to sterilization. In experiments with polyhydric compounds, the additives were added to the milks prior to processing.

In several experiments with phosphatides, the milks were modified with synthetic serums to yield products with a weight ratio between fat and protein of 4.5 to 2.4 in one experiment (see Table 10) and 5.0 to 1.9 in another (see Table 11). The serum diluents had the same pH as milk and approximately the same ionic strength as milk serum.

Materials. Commercially available condensed phosphates under various trade names were

[1] The use of trade names is for the purpose of identification only, and does not imply endorsement of the product or its manufacturer by the U. S. Department of Agriculture.

TABLE 1

Some properties of the polyphosphate additives

Source	Ratio Na_2O/P_2O_5	Average no. of P atoms per chain (calculated)	Average no. of P atoms per chain (experimental)	Weight phosphorus mg per g (experimental)
Laboratory prepared	1.05	40.0	31.9	303
Laboratory prepared	1.18	11.0	10.8	289
Laboratory prepared	1.25	8.0	7.8	286
Laboratory prepared	1.30	6.7	6.7	286
Commercial [a]	.....	.....	4.8	263
Commercial [b]	2.0	2.0	.....	139
Commercial [c]	.....	.....	7.0	286
Commercial [d]	1.0	.....	.....	258
Commercial [e]	.....	3.0	.....	156
Commercial [f]	1.67	3.0	.....	252

[a] Sodium polyphosphate glass.
[b] Sodium pyrophosphate + 10 H_2O.
[c] Sodium hexametaphosphate glass.
[d] Sodium tetrametaphosphate + 4 H_2O.
[e] Adenosine triphosphate.
[f] Granular sodium tripolyphosphate.

obtained from a number of manufacturers. The commercial compounds and crystalline sorbitol, dextrose, lactose, sucrose, and $MnSO_4 \cdot H_2O$ were of the highest quality. Dextran was obtained through the courtesy of Dr. Allene Jeanes of the Northern Utilization Research and Development Division, U. S. Department of Agriculture, Peoria, Illinois. Purified lecithin and cephalin were prepared from vegetable lecithin according to the instructions of Thornton et al. (17) and Folch (4), respectively. The alcohol-insoluble phosphatides were purchased from Central Soya Company [1] and were specified to contain 4.0% chemical lecithin, 28.5% chemical cephalin, 55.0% inositol phosphatides, 4.0% soybean oil, and 8.5% miscellaneous.

Table 1 lists the commercially available polyphosphates which were tested and some of their properties. In addition, a number of chain polyphosphates were prepared in the laboratory from mixtures of monobasic and dibasic phosphates according to a method outline by Van Wazer and Holst (23). The average number, $\bar{n}$, of phosphorous atoms per chain and the weight of phosphorus per gram were determined by titration (19). This information is also contained in Table 1.

RESULTS AND DISCUSSION

Salient features of the data on the effect of polyphosphates, manganous sulfate, polyhydric compounds, and phosphatides, are contained in Tables 1-6, 7, 8, and 9-11, respectively.

Theoretical considerations. As a first step in this study, a working hypothesis was formu-

TABLE 2

Storage life of skimmilk concentrates (28%) solids with and without added sodium polyphosphate glass (4.8 phosphorous atoms on an average per chain)

Additive concentration	Viscosity before sterilization	Storage time in days										Min. viscosity	Storage life [b]
		0	8	16	21	28	35	49	69	91	130		
g per 100 g milk solids	(centipoises)	Viscosity at 30 C centipoises										centipoises	days
None	7.0	12.0	8.4	19.2	gel							8.4	16
0.14	7.5	13.4	8.2	9.1	20.0	gel						8.2	20
0.29	8.0	11.4	7.0	6.8	7.2	10.2	33.8	gel				6.7	32
0.56	8.5	11.4	6.7	6.5	6.3	6.4	6.3	6.4	7.7	16.9	gel	6.3	89
0.56 [a]	12.1	22.9	8.9	8.5	8.5	8.5	8.5	8.5	9.2	11.6	19.3	8.5	128

Sterilization temperature—137.4 C.
Sterilization time—5 sec.
[a] Polyphosphate added after sterilization.
[b] Time in days required for viscosity to attain a value equal to twice the minimum value.

lated which will be discussed briefly. The existence in equilibrium of two micellar species is postulated, reactive (denatured) and unreactive. The tendency of casein monomers to pass in and out of the micelle to a degree depending on the amount of cross-linking or stabilizing agents (among which are calcium ions) present in the micelle gives rise to a reactive form consisting of a core with protruding monomer filaments. As the temperature is raised, calcium ions tend to enter the micelle and stabilize it further by drawing together the monomer filaments, thus giving rise to an unreactive smooth and compact form characterized by the absence of protruding filaments. Denaturation reactions with negative temperature coefficients have been observed (7). This form as the temperature is lowered tends to revert to the reactive one at a rate and to a degree depending on the equilibrium temperature. However, prolonged heating stabilizes the unreactive form against reversion. Unreactive micelles behaving as emulsoid particles, although quite stable at storage temperature, interact at elevated temperatures through the agency of polar groups on their surfaces mediated by calcium ions. Activated micelles, on the other hand, behave as if they were the centers of long-range attractive forces, and it is interaction mediated by calcium ions between the monomer filaments belonging to these micelles which leads to structure formation. Ad-

TABLE 3

Results of laboratory-scale experiments showing how the storage life of high-temperature–short-time sterilized whole milk concentrate (26% solids) is extended with added amorphous sodium polyphosphate (4.8 P atoms per chain) and shortened with added monophosphate salts

Additive	Viscosity at 86 F before sterilization	Viscosity after sterilization	Minimum viscosity	Storage life at 86 F
	(centipoises)			*(days)*
None	4.4	4.5	4.1	107
Monophosphate buffer salts	4.4	4.5	3.9	55
Amorphous sodium polyphosphate (4.8 phosphorous atoms per chain)	4.4	4.3	3.3	274

Ratio between solids and fat	3.25:1
Homogenization (before forewarming) at	2,500 psi and 160 F
Forewarming for 15 sec at	280 F
Concentration to solids concn. of	26%
Sterilization for 15 sec at	280 F
Buffer salt (g per 100 g 12.6% fluid milk)	0.053
Polyphosphate (g per 100 g 12.6% fluid milk)	0.049
Storage life = time required for viscosity to reach a value equal to twice minimum	
Viscosity in centipoises of polyphosphate containing concentrate after 274 days	3.3

TABLE 4

Storage life of whole milk concentrates (36% solids) with and without added sodium polyphosphate glass (4.8 phosphorous atom on average per chain)

Additive concentration	Viscosity before sterilization	Viscosity after sterilization	Minimum viscosity	Time to reach minimum viscosity	Storage life [a] at 30 C
(g per 100 g milk solids–nonfat)		*(centipoises)*		*(days)*	*(days)*
None	14.5	35.3	31.0	4	9
0.59	19.3	29.5	21.8	4	91
0.66	19.5	27.5	19.8	4	120
0.74	19.8	26.5	18.6	6	150

Sterilization temperature—137.4 C.
Sterilization time—5 sec.
[a] Time required for viscosity to reach a value equal to twice the minimum value.

ditives capable of diffusing into the micelle and capable of forming cross-linkages between protein monomers would have the same effect as heat, tending to bring about a retraction of monomer strands, thus compacting the micelle and shifting the equilibrium in favor of the unreactive form.

Stabilization by polyphosphates. The polyphosphates with respect to their value as additives for stabilizing high-short concentrated milk products possess the following important properties:

1. A high proton donor capacity per molecule of the corresponding acids.
2. A marked stability against hydrolysis and reversion to the monomeric phosphates.
3. A metal complexing capacity (20).
4. Physiological and pharmacological properties consistent with their use in foods.

Well known among protein chemists is the high proton donor capacity of the condensed phosphoric acids which permits them to function as protein precipitants—an interaction involving the positively charged groups on the protein and negatively charged polyphosphate anions (8).

Interactions of this character at the normal pH of concentrated milk result in a building

TABLE 5

Influence of some commercially available polyphosphates on storage life of a HTST sterilized milk concentrate (28.2% milk solids).[a] Sterilization temperature and time, 137.4 C and 5 sec

Additive	Additive concentration[b]	Viscosity before sterilization	Viscosity after sterilization	Minimum viscosity	Time to reach min. viscosity	Storage life at 30 C
	(g per 100 g milk solids)	*(centipoises)*			*(days)*	*(days)*
None	.....	9.0	12.8	11.0	4-6	11
Pyrophosphate	0.61	13.2	12.6	8.9	4-6	33
Tripolyphosphate	0.56	11.8	12.9	8.3	4-6	59
Adenosine triphosphate	0.93	9.9	14.5	11.1	4-6	80
Polyphosphate $\bar{n} = 4.8$	0.54	10.8	12.6	8.1	4-6	90
Hexametaphosphate	0.50	10.2	12.8	7.5	4-6	148
Tetrametaphosphate	0.55	9.0	11.6	7.9	5-8	159

[a] Skimmilk used in this experiment had been stored at 4 degrees for 1 wk. It was derived from a fresh milk as that used to obtain data shown in Table 6.

[b] Concentration refers to weight of additives in anhydrous state. Additives contain same number of available orthophosphate groups.

TABLE 6

Influence of polyphosphate glasses, and orthophosphates on storage life of a HTST sterilized skimmilk concentrate containing 28.2% milk solids. Sterilization temperature and time, 137.4 C, and 5 sec, respectively

Additive	Additive concentration	Viscosity before sterilization	Viscosity after sterilization	Minimum viscosity	Time to reach min. viscosity	Storage life
Av. no. of P atoms per chain ($\bar{n}$)	*(g per 100 g milk solids)*	*(centipoises)*			*(days at 30 C)*	*(days at 30 C)*
None[a]	.....	9.0	11.1	9.4	4-6	16
A ($\bar{n} = 1$)[b]	0.13	9.8	14.6	10.2	4-6	9
B ($\bar{n} = 6.7$)	0.29	9.7	9.2	6.2	4-6	85
B ($\bar{n} = 6.7$)	0.48	10.2	9.9	6.7	4-6	135
B ($\bar{n} = 6.7$)	0.63	11.7	10.4	6.7	4-6	148
C ($\bar{n} = 7.8$)	0.29	9.7	10.8	6.8	4-6	85
C ($\bar{n} = 7.8$)	0.48	10.4	10.7	7.1	4-6	151
C ($\bar{n} = 7.8$)	0.63	11.7	10.4	6.6	4-6	170
D ($\bar{n} = 10.8$)	0.29	9.5	10.9	7.4	4-6	103
D ($\bar{n} = 10.8$)	0.47	10.3	11.3	7.1	4-6	167
D ($\bar{n} = 10.8$)	0.65	11.5	11.5	7.2	4-6	180
E ($\bar{n} = 31.9$)	0.28	9.5	10.6	7.3	4-6	109
E ($\bar{n} = 31.9$)	0.45	10.4	12.0	7.5	4-6	169
E ($\bar{n} = 31.9$)	0.62	12.2	13.1	7.8	4-6	180

[a] Control sample for B, D, C, and E, but not for A; Control sample for A contained 29.4% solids and had a storage life of 13 days.

[b] Additive consists of a mixture of orthophosphates, pH 6.5.

up of the net negative charge on the micelles, an effect which conduces to an expansion of the micelles and to swelling. Expansion is engendered by electrostatic repulsion, and swelling by osmosis in view of the Donnan effect. The increase in hydrodynamic volume is con-

TABLE 7

Life of skimmilk concentrates (28.3% milk solids) with and without added manganous sulfate

Concentration of $MnSO_4 \cdot H_2O$	Viscosity before sterilization	Viscosity after sterilization	Minimum viscosity	Time to reach minimum viscosity	Storage life at 30 C [a]	Remarks
(g per 100 g milk solids)	*(centipoises)*			*(days)*	*(days)*	
0	9.0	14.3	10.1	4	14	
0.135	8.4	13.8	9.9	8	26	
0.27	8.0	15.6	10.6	11	56	Concentrate exhibits plastic flow and thixotropy at end of storage life
0.54	7.6	49.0	15.3	13	120	Concentrate exhibits plastic flow and thixotropy at end of storage life

All viscosity measurements at 30 C.
Sterilization temperature 280 F; time 5 sec.
[a] Time in days required for viscosity to attain a value equal to twice the minimum value.

TABLE 8

Influence of various sugars and sorbitol on storage life of HTST sterilized skimmilk concentrates (19.2% milk solids). Sterilization temperature 137.4 C, sterilization time 15 sec

Additive	Additive concentration	Viscosity before sterilization	Viscosity after sterilization	Minimum viscosity during storage	Storage life at 30 C
	(g per 100 g prod.)	*(centipoises)*			*(days)*
None (control)	.....	2.8	3.0	2.6	111 [a]
Lactose	9.6	3.1	3.4	3.4	180 [b, c]
	17.5	4.2	4.4	4.4	192 [b, d]
	24.2	5.3	5.7	5.7	110 [b]
Dextrose	9.6	3.4	3.4	3.4	175 [b]
	24.2	5.2	5.3	5.3	249 [b]
None (control)	.....	2.8	3.1	2.5	98 [e]
Sucrose	9.6	3.5	3.8	3.4	190 [b]
	17.5	4.5	4.5	4.5	315 [b]
	24.2	5.7	5.7	5.7	144 [b]
Sorbitol	9.6	3.2	3.1	3.1	172 [b]
	17.5	4.0	3.8	3.8	360 [b]
	24.2	5.1	4.8	4.8	607 [b]

[a] Coagula visible after 124 days; marked coagulation after 134 days.
[b] Thickening but no coagulation observable during storage period.
[c] Viscosity after 210 days—12 centipoises.
[d] Viscosity after 350 days—15 centipoises.
[e] Coagula visible after 109 days; marked coagulation after 115 days.

sistent with an increase in viscosity. The effect of charge in concentrated milks is particularly striking when concentrations of polyphosphate greater than 1 g per 100 g milk solids in 3 to 1 milks are employed, and in such concentrates, a marked thickening effect is observed. At lower concentrations, such as those reported in this paper, the thickening effect which is small in the unsterilized product is more than compensated for by effects which take place in

TABLE 9

Effect of various phosphatide preparations on storage life of concentrated homogenized [b] milk (26% solids) sterilized at 137.4 C for 15 sec

Sample	Apparent viscosity			Storage period required for:		
	Before sterilization	After sterilization	Minimum during storage	Onset of viscosity increase	Viscosity to reach twice minimum	Cream layer after 70 days [b]
	(centipoises)			*(days)*	*(days)*	
Concentrated milk	4.9	16.4	7.2	38	65	6
Concentrated milk plus 0.33% chemical lecithin [c]	4.9	13.1	6.6	41	88	0
Concentrated milk plus plus 0.33% chemical cephalin [d]	4.9	14.4	6.1	41	88	0
Concentrated milk plus 0.33% crude animal lecithin	4.9	16.1	6.2	41	85	0

[a] Samples stored in capillary tubes and held quiescent in vertical position at 30 C.

[b] Milk homogenized in two stages and recycled to yield a product with homogenization index of 87%.

[c] Phosphatidyl choline.

[d] Phosphatidyl ethanolamine.

TABLE 10

Improvements of storage life effected by addition of cephalin to modified milk [a] concentrates (26% solids) sterilized at 137.4 C for 15 sec

Sample	Apparent viscosity			Storage period required for:		Remarks
	Before sterilization	After sterilization	Minimum during storage	Onset of viscosity increase	Viscosity to reach twice minimum	
	(centipoises)			*(days)*	*(days)*	
Unforewarmed concentrate, no cephalin	3.5	Forms gel	Forms gel	0.0		Coagula visible after sterilization
Unforewarmed concentrate, with added cephalin	4.6	11.9	11.9	99	126	Highly thixotropic structure after sterilization
Forewarmed concentrate, no cephalin	3.0	4.3	4.3	49	77	
Forewarmed concentrate, with added cephalin	3.7	5.4	5.0	400	400	Extremely fragile structure forms in some samples

Additive-containing milks contain 0.3 g cephalin per 100 g unconcentrated milk.

Viscosity measured after sample had been inverted twice.

[a] Modified milks (ratio between fat and protein—4.5 to 2.4) prepared by blending cream and skimmilk with a synthetic serum, pH 6.5, ionic strength 0.075 containing per liter: 50 g lactose, 3.43 g $Na_2HPO_4 2H_2O$, 0.75 g citric acid, 2.80 g KCl and NaOH to bring pH to 6.5. Milks homogenized at 65 C in two stages—7,500 and 500 psi.

TABLE 11

Influence of alcohol-insoluble phosphatides on storage life of modified milk [c] concentrates sterilized at 137.4 C for 15 sec

Sample	Phosphatide concentration	Viscosity before sterilization	Viscosity after sterilization	Minimum viscosity	Storage [a] life	Remarks
	g per 100 g concentrate	(centipoises)			(days)	
Modified skimmilk concentrates	0	2.1	2.1	1.9	162	Marked body deterioration after 179 days; marked coagulation
Modified whole milk concentrate	0	3.0	5.7	5.3	88	Stirred-out viscosity[b] at end of 137 days —45 centipoises
Modified whole milk concentrate	0.2	3.3	4.0	3.8	122	
Modified whole milk concentrate	0.4	3.5	3.9	3.8	157	
Modified whole milk concentrate	0.6	3.6	4.0 3.9	3.9	175	Soft, homogenous, highly thixotropic body prevails after end of storage life. Stirred-out viscosity after 218 days —13.3 centipoises

[a] Time in days for viscosity to reach a value equal to twice minimum vaule observed during storage.

[b] Stirred out by alternately freezing sample in dry ice–acetone bath and thawing.

[c] Modified skim prepared by blending 28 lb milk with 10.6 lb of a synthetic serum, pH 6.5 ionic strength, 0.075 containing per liter: 50 g lactose, 0.46 g citric acid, 2.80 g KCl, 1.46 g NaCl, and 0.32 g NaOH. Modified whole milks prepared by mixing 9.5 lb, modified skim with 0.5 lb butter oil containing graded concentrations of phosphatides. Dispersions of phosphatides in water unstable. Hence, they were dissolved in butter oil which had been separated from butter at 50 C and clarified at 120 C. Milks homogenized at 65 C in two stages—7,500 and 500 psi.

milk concentrates during sterilization and storage.

In milks containing cyclic phosphates, reversion to a thinner body takes place largely during storage. The stabilizing effect observed during sterilization of concentrates containing linear condensed phosphates may be attributed to the complexing of calcium, and thus the chain polyphosphates, like the orthophosphates, function as stabilizers against heat coagulation. The augmented attenuation of body during storage may be postulated to result from a shift in equilibrium favoring intramicellar stabilization as opposed to intermicellar interaction. The thinning out of body is particularly pronounced in the concentrate containing the cyclic phosphate. Inasmuch as the cyclic phosphates do not form strong complexes with calcium, the entire decrease in viscosity to a value considerably lower than that of the control milk before its sterilization can reasonably be attributed to a type of intramicellar interaction involving volume contraction.

The thickening in concentrates containing high concentrations of polyphosphate is reversible in character, for on diluting the concentrates to the concentration of normal milk, the viscosity and smooth texture of the original milk is restored (data are not recorded in this paper). Thickening brought about by the addition of large quantities of polyphosphates increases during storage of the concentrate, although structure formation is delayed.

Antigelation effectiveness per phosphorous atom increases with increasing chain length of the linear polyphosphates, an effect which is more pronounced among the polymers of short-chain length. This effect may be associated with the greater effectiveness of the long-chain polyphosphates compared to the shorter-chain compounds as protein precipitants and, hence, to their greater affinity for proteins (8).

Adenosine triphosphate is more effective than the corresponding sodium tripolyphosphate. It appears, therefore, that the adenosine moiety augments the affinity for proteins of the polyphosphate grouping. In support of this observation are the observations of Leviton and Pallansch on the binding of riboflavin and riboflavin phosphate (11).

Influence of polyphosphate concentration. Storage life of HTST sterilized milk concentrates is significantly increased in the presence of added polyphosphate. The increase varies with additive concentration, and the rate of increase is more pronounced at the lower concentrations. Employed at a concentration level of approximately 0.05% based on the original milk, the effect of the polyphosphates with more than four phosphorus atoms per chain, is to prolong the storage life at 30 C of 3 to 1 milk concentrates from 2 or 3 wk to many months and, correspondingly, the storage life of two to one concentrates from three or four months to more than nine months. Limiting the effectiveness of the polyphosphates is their slow conversion to the orthophosphates. Inasmuch as the orthophosphates tend to accelerate the gelation rate (see Table 6), this net result of the hydrolysis of polyphosphates would be magnified to the extent that the orthophosphates are formed. The greater the chain length of the polyphosphates, the slower would be the hydrolysis to and subsequent accumulation of orthophosphates in milk concentrates, and the less noticeable would be the contribution of increasing orthophosphate concentration to a reduction in storage life. The cyclic phosphates, because they resist hydrolysis to the chain polyphosphates to an appreciable degree have, when employed as additives, the advantage of a greater over-all stability (compared to the corresponding linear compounds) against conversion to orthophosphates (1).

Possibly another limiting factor with respect to the effectiveness of the linear polyphosphates is their complexing affinity for calcium and similar metal ions. Inasmuch as calcium ion itself possesses antigelation activity, the net effect of complex formation would be the reduction in effective concentrations of both calcium and polyphosphate (12). The cyclic phosphate, unable to complex with Ca^{++} to the same degree as the linear polyphosphates, at least not until it has been hydrolyzed to the corresponding linear polyphosphate, would not be subject to the aforementioned limitation.

There is a strong temptation to associate the behavior of the polyphosphates with their calcium-complexing capacity leading to the sequestration of calcium ions. In conflict with this idea, however, are these observations: added calcium augments storage life, whereas added orthophosphates diminish it; the cyclic phosphate with its relatively weak complex-forming capacity is quite effective in retarding gelation. It appears, therefore, that the ability to complex with metals is a property of the polyphosphates which, if related to its antigelation function at all, comes into play as result of intramicellar interaction involving the formation of a calcium caseinate–condensed phosphate complex.

Suitability of polyphosphates as food additives. The addition to evaporated milk of stabilizing salts to the extent of 0.1% by weight of the finished product has been legalized in the United States. The use of polyphosphates as food additives is not novel. Their use to the extent of 3-3.5% in processed cheese appears to be sanctioned in all processed cheese producing countries. Kiermeier and Möhler not only have tabulated the quantities of added condensed phosphates in various food products (9), but have also examined the question of the suitability of these substances as food additives, referring to the work of Schreier and Nöller (15).

Stabilization by manganous salts. Calcium salts stabilize slightly and orthophosphates destabilize high-short sterilized milk concentrates against gelation during storage. These salts function in the opposite sense during the sterilization of milk, the calcium salts acting to destabilize and the orthophosphates to stabilize against heat coagulation.

The effect of divalent manganese compared with the action of divalent calcium ions is quite large. The 8-9 fold extension of storage life brought about by its addition (0.54 g per 100 g milk solids) to 3 to 1 sterile skimmilk concentrate is of the same order of magnitude as that observed when the higher polyphosphates at the same concentration level are added to milk. The prolongation is roughly proportional to the concentration of added Mn^{++}.

Mn^{++}, when added to the concentrate before sterilization, brings about a significant decrease in viscosity in proportion to the quantity added. In this respect, it functions in a sense opposite to that observed when polyphosphates are added to milk. Manganese is known to form complexes with casein, and to the extent that it does, it would lower the negative charge on the micelles in milk; a certain amount of contraction would be expected as well as a reduction in the electroviscous effect, and a loss of

water in consequence of the Donnan effect. It is doubtful, however, that the contraction in micellar volume arising from these effects would account entirely for the decrease in viscosity, inasmuch as the charge on the protein is in virtue of bound calcium already reduced to the point where the Donnan effect is small. It is not unreasonable, therefore, to account for the observed decrease in viscosity by assuming that the concomitant contraction in hydrodynamic volume is a consequence of cross-linking.

Sterilization is attended by an increase in viscosity, an increase which remains nearly independent of Mn^{++} at low levels of concentration, and which rises abruptly when the concentration of added $MnSO_4 \cdot H_2O$ reaches 0.54 g per 100 g milk solids.

Much of the observed increase in viscosity disappears during storage at a rate which decreases as the concentration of $MnSO_4$ is increased. If the increase in viscosity attending sterilization can be ascribed to the reversible formation of aggregates of modified micelles mediated by manganous ions, the subsequent thinning out of body may be ascribed to a reversal of this process, and age-thickening to the slow internal change in the structure of the micelles followed by aggregation of the changed micelles.

Judged solely from the extent of their action in prolonging storage life with respect to gelation, manganous salts are as efficient as the polyphosphates. The amount of manganese added to a pint of milk in the preparation of HTST sterilized milk concentrates would be far in excess of the amount of manganese found in human rations (16).

Possessing a number of valence levels manganese salts catalyze the formation of hydroperoxides in the oxidation of fatty acid esters in anhydrous nonpolar solvents (18). Inasmuch as this reaction is inhibited in the presence of polar solvents, an effect attributed to the formation of solvates, it may very well be that in aqueous solution steric hindrance would prevent Mn^{++} from functioning as a catalyst.

It has been found that manganese in butter exists in true solution in the serum phase, whereas copper and iron are bound to the colloidal constituents (proteins and phosphatides) of the serum (3). Thus, it is reasonable to assume that the effect of manganese in the oxidation of milk fat would not parallel that of copper and iron.

Garrett (5) added 0-0.12 mM $MnCl_2$ to a liter of milk and 0.06 mM Cu^{++}, and found that the intensity of the oxidized flavor which developed in 96 hr was reduced from a maximum of five arbitrary flavor units (in the absence of added Mn^{++}) to zero. Added $MnCl_2$, unlike added copper salts, did not influence the development of oxidized flavor adversely, nor did it catalyze the oxidation of ascorbic acid.

It appears, in view of the aforementioned evidence, that the evaluation of manganous salts with respect to whatever flavor changes they may engender in milk concentrates must await further investigation. Concerning the utility of such salts in retarding gelation, there can be no question.

Stabilization by polyhydric compounds. To produce any measurable effect in milk concentrates, polyhydric compounds compared with manganous salts and polyphosphates must be used in high concentrations. Added to 2 to 1 sterile milk concentrates to the extent of 9.6 g per 100 g product the polyhydric compounds lactose, sucrose, dextrose, and sorbitol extended storage life 1.7 to 2 fold. The increase in viscosity (approximately 10%) brought about by the addition of these polyhydric compounds to the unsterile concentrates, reflecting as it does the increase in the viscosity of the continuous phase, is in no way commensurate with the increase in storage life. It appears, therefore, that the added storage life is not a consequence of a diminished micellar diffusion rate (the rate would be inversely proportional to the viscosity) but is rather a consequence of certain properties possessed in common by the polyhydric compounds.

Present in milk in higher concentrations, these compounds begin to exhibit differences in behavior which reflect differences in molecular groupings and structure. Thus, the disaccharides lose much of their effectiveness as the concentration in 2 to 1 milk is increased beyond 17.5 g per 100 g product. Sorbitol becomes increasingly effective as its concentration is increased. An interesting feature associated with the use of polyhydric compounds is that in their presence, sedimentation is visibly retarded, and flocculation such as one may observe in control milks in the late stages of age-thickening is largely absent.

It would be difficult to interpret the action of polyhydric compounds without assuming that interaction between these compounds and micellar proteins is involved. Such interaction is indicated in the results with dextran (data not included in this paper). Sterilization of dextran containing concentrates results in an appreciable increase in viscosity not shared by the control concentrates.

Stabilization by phosphatides. The participation of the lipid phase in coagulation phenomena has been the subject of a previous report in this series of publications (13). The quasi-inert behavior of this phase with respect to gelation was considered to indicate the existence of two diametrically opposed tendencies, one prompting the fat globules to behave as oriented protein particles, and the other, as oriented phospholipid particles. Behaving as protein, the lipid phase would tend to promote coagulation (a concentration effect) and, behaving as phosphatide particles, the fat globules would tend to hinder the development of graininess and structure formation by interfering with the free diffusion of interacting protein particles. Many milks behave as if they were out of balance with respect to the composition of the interfacial region. Modified milks rich in fat and relatively deficient in proteins may be prepared which show this imbalance to an exaggerated degree. Such milks respond to corrective measures and improved heat and storage stability is realized, as the results in Tables 9-11 show, if phospholipids are added to compensate for compositional deficiencies in the interfacial region.

The experiments in which the protein content of the milk was reduced by means of a synthetic serum and the fat content was correspondingly increased, illustrates strikingly how the fat rather than the protein phase may limit both the heat and storage stability of concentrated milks.

The possibility of producing highly stable milk concentrates with a high ratio between fat and protein is indicated. Such concentrates would have the same total solids as conventional HTST sterile concentrates, and would be richer in milk flavor. Much more work remains to be carried out in this connection, however, for although structure formation requires a definite minimum concentration of interacting elements, it is unlikely that aggregate formation is likewise limited.

The two purified phosphatides, lecithin and cephalin, appear to be equally effective, although marked deterioration in body was less noticeable in the cephalin-containing product. Equilibrium conditions in the interfacial region are probably reached slowly, if at all, and consequently variables such as rate and duration of heating and cooling, and the manner in which additives are incorporated (whether in the aqueous or in the fat phase, for example) influence results. It would be logical to incorporate additives in the lipid phase, for in this way labile substances are protected against hydrolysis and precipitation and diffusion into the interphase is expedited.

Forewarming, it appears, can play a part in the manufacture of HTST concentrated milk products no less important than the part it plays in the manufacture of conventional evaporated milk (see Table 11). Thus, forewarming serves to stabilize HTST milk concentrates against heat coagulation, a point of importance (notwithstanding the general impression that heat stability is not an important factor in HTST sterilization), and it serves to prolong storage life by retarding the developments of structure.

Footnote. Following the completion of the studies reported in this paper, the very recent and pertinent paper by Hoff et al. on irradiation induced gelation became available (6). Milk concentrates sterilized by ionizing radiation, it appears, become quite unstable toward heat and coagulate instantly at 90 C. These concentrates are also quite labile during storage and acquire within days a gel-like structure. Inasmuch as HTST sterile milk concentrates, unlike irradiated concentrates, are not instantaneously coagulated at 90 C, it would appear that irradiation-induced gelation is unrelated to gelation in HTST sterilized milk. However, in view of the observations of Hoff et al. that manganese salts and polyphosphates are effective inhibitors of irradiation-induced gelation, and in view of the observations recorded in this paper on the effects of these compounds in HTST sterilized milk concentrates, it appears likely that the gelation mechanisms are related.

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